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Renal cancer genetics: von Hippel Lindau and other syndromes

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ABSTRACT There have been significant advances in our understanding of the genetic basis of renal carcinogenesis. In particular, research in the last five years has demonstrated a central role for the inactivation of the von Hippel-Lindau gene by mutation or hypermethylation in the formation of the conventional type of renal cell carcinoma. The von Hippel-Lindau syndrome is characterised by germ-line inactivating mutation whereas sporadic renal carcinoma is associated with somatic mutations. Tumour formation is accompanied by loss of the remaining wild-type allele. The biology of the von Hippel-Lindau gene and its normal function continued to be unravelled but a role has been demonstrated for it in the regulation of gene transcription, the regulation of oxygen-dependent genes and their expression and the control of tumour angiogenesis acting via the vascular endothelial growth factor. Another form of familial renal cancer, the hereditary papillary renal cell carcinoma, has been shown to be consequent upon activating mutations of the c-met proto-oncogene. The genetic data continue to enhance our understanding of the biology of this common set of neoplasms.

KEY WORDS: *renal carcinoma, von Hippel Lindau syndrome, hereditary papillary renal cell carcinoma*

Introduction

Significant advances in our understanding of the genetic basis of renal carcinogenesis have led to a re-evaluation of the classification of these tumours. At the recent Heidelberg and Mayo Clinic Conferences a new classification was formulated (Bostwick *et al.*, 1997; Kovacs *et al.*, 1997). Although this classification uses histopathological criteria, it is based on evidence which shows a strong correlation between histology, clinical behaviour, epidemiology and the underlying genetic abnormalities. The classification of the major adult renal epithelial malignancies is summarised in Table 1. The conventional type of renal cell carcinoma is the most frequently encountered in the clinical practice, accounting for 70% of cases in surgical series and is consistently associated with losses on the short arm of chromosome 3 (Fleming, 1997). Less common, accounting for 15% of cases in surgical series, is papillary renal cell carcinoma. This tumour shows a different epidemiological pattern and an association with trisomies of chromosomes 7 and 17 and loss of Y chromosome in males (Kovacs, 1993). Chromophobe carcinomas and collecting duct carcinomas are much less frequent and the chromosomal and molecular genetic data on these, consequently, less accurately defined.

von Hippel Lindau syndrome and conventional type renal cell carcinoma

The conventional type of renal cell carcinoma is characterised by cuboidal clear cells arranged in cords or trabeculae and supported by a rich capillary meshwork. The tumours are often well circumscribed from the adjacent renal cortex, in this respect less infiltrative than other cancers such as breast, colorectal and pancreatic, but are highly vascular showing intratumoural haemorrhage. Their association with the von Hippel Lindau syndrome has been recognised for some time. This syndrome is an autosomal dominantly inherited cancer predisposition syndrome. It affects approximately 1:35,000 individuals who suffer from a greatly increased risk of the development of a variety of tumours including CNS and retinal haemangioblastoma, phaeochromocytoma and renal cell carcinoma often preceded by renal cystic disease and atypical cyst formation (Maher *et al.*, 1990). The development of the renal cell carcinomas is a major prognostic feature in the von Hippel Lindau syndrome. It

Abbreviations used in this paper: VHL, von Hippel-Lindau syndrome; CNS, central nervous system; RTPCR, reverse transcriptase polymerase chain reaction; VEGF, vascular endothelial growth factor.

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has been known for a number of years that the gene associated with the von Hippel Lindau syndrome was located on chromosome 3p25 (Kovacs *et al.*, 1988). The gene was cloned by a large international group using a positional cloning strategy (Latif *et al.*, 1993). The gene is composed of three exons encoding two alternative splice mRNA isoforms of 6 Kb and 6.5 Kb. The alternative sequences involve the inclusion or exclusion of exon 2. The gene has a unique sequence with no similarity to known proteins which would assist in assigning a function. A variety of different mutations of this gene have been found in kindreds suffering from the von Hippel Lindau syndrome, but the development of tumours has been shown to depend upon loss of the remaining normal wild type allele. Following the identification of the von Hippel Lindau gene three groups performed mutational analyses of the *VHL* gene in sporadic renal cell carcinomas (Foster *et al.*, 1994; Gnarra *et al.*, 1994; Shuin *et al.*, 1994). The findings of these groups from America, Japan and Europe were broadly similar. Somatic mutations of the *VHL* gene were identified in up to 65% of renal cell carcinomas of the conventional type but were not found in papillary or chromophobe renal cell carcinomas. The tumour formation was almost uniformly accompanied by loss of the wild type allele usually as a consequence of loss of a significant proportion of chromosome 3p. The mutations were of a variety of different types most frequently, but not exclusively, frame-shift mutations. In sporadic renal cell carcinoma mutations were extremely rare in the first 120 codons but were found throughout the remainder of the gene. A comparison of the location of germ line versus somatic mutations reveals an interesting difference. Germ line mutations associated with the von Hippel Lindau syndrome rarely affect exon 2, whereas this exon is affected in greater then 40% of the somatic mutations seen in conventional renal cell carcinoma (Linehan *et al.*, 1995). The reason for this difference is unclear but one possibility is that loss of functional exon 2 with a consequent change in the ratio of the two alternative splice isoforms may be a critical developmental defect. In addition to mutational alteration of function, it has recently been demonstrated that *VHL* gene expression may be reduced by hypermethylation in some cases, possibly as many as 15% of sporadic renal cell carcinomas (Herman *et al.*, 1994). In these instances the transformed phenotype *in vitro* can be reversed by methylation inhibitors. The mutational analysis and the evidence of hypermethylation suggests that somatic inactivation of the *VHL* gene occurs in up to 80% of conventional renal cell carcinomas and that loss of the wild type allele is a critical event in tumorigenesis. Therefore, the *VHL* gene shows a pattern of behaviour characteristic of a tumour suppressor gene.

Analysis of the expression of the *VHL* gene and its protein product during embryogenesis and in adult life have been performed using *in situ* hybridisation, Northern blot analysis and immunocytochemistry (Kessler *et al.*, 1995; Richards *et al.*, 1996). In these experiments within the kidney, *VHL* mRNA and protein are not expressed in the metanephric blastema or early nephrons but rather are differentially expressed at high levels within the growing renal tubules, particularly within the elongating loops of Henle and, to a lesser extent, the collecting ducts. Some reactivity was also found in the proximal and distal tubules but at a lower level. Quantitative RTPCR analysis of the developing kidney shows that the two isoforms are expressed with the larger isoform (Richards *et al.*, 1996), composed of exons 1,2 and 3, expressed at approximately double the level of the smaller isoform. Renal tubules cultured *in vitro* expressed predominantly the larger isoform with minimal expression of the smaller. High levels of expression were also identified in the developing gonads, lung and central nervous system. Interestingly, although the VHL syndrome is characterised by the development of phaeochromocytomas, significant levels of expression were not found in the fetal adrenal gland. However, phaeochromocytomas develop from the adrenal medulla and medullary cells may be under-represented in analysis of the fetal adrenal.

The functions of the VHL protein are just beginning to be elucidated. It has been shown that the protein binds to the B and C subunits of the multimolecular protein complex elongin (Duan *et al.*, 1995). This binding with the VHL protein blocks the assembly of the elongin complex containing the catalytic A unit. Thus VHL protein binding negatively regulates elongin function. Elongin is part of the RNA transcription elongation machinery involving RNA polymerase 2. This suggests that one possible mechanism for action for VHL is to negatively regulate gene transcription. However, it appears difficult to reconcile this apparently generalised suppression of RNA transcription with the more specific features which may be seen in the von Hippel Lindau syndrome and in renal cancer. The VHL/elongin BC complex (VBC) has also recently been shown to bind to the human homologue of CUL-2 protein involved in the regulation of cell cycle entry (Pause *et al.*, 1997).

Whatever the precise mechanism of action at the molecular level of the von Hippel Lindau protein, recent evidence has suggested it is active in negatively regulating the expression of the angiogenesis factor, vascular endothelial growth factor (VEGF) (Siemeister *et al.*, 1996). This potent angiogenesis factor, considered to be the most important in tumour angiogenesis by renal cell carcinoma, is expressed at high levels in conventional type renal cell carcinomas which are associated with VHL mutation. In *in vitro* experiments the re-introduction of a normal VHL gene into cell cultures of conventional type renal cell carcinoma has led to a reduction in the level of VEGF expression by these tumours. This has suggested the hypothesis that one of the modes of action during tumour development and progression of VHL mutation is to fail to block this angiogenesis factor leading to enhanced tumour angiogenesis. VEGF is a hypoxia responsive gene and although, initially, it was suggested that VHL mutation led to increased transcription of this gene, recent experiments have shown that it is more accurate to conclude that mutation in the VHL gene uncouples VEGF expression from the hypoxia sensing

TABLE 1

NEW CLASSIFICATION OF ADULT RENAL EPITHELIAL TUMOURS

Benign:
Papillary adenoma
Metanephric adenoma
Oncocytoma
Malignant:
Renal cell carcinoma conventional (clear cell) type
Papillary renal cell carcinoma
Chromophobe renal carcinoma
Collecting duct carcinoma
Unclassifiable

apparatus within the cell possibly by influencing RNA half-life (Gnarra *et al.*, 1996).

Transgenic knock-out mice with targeted deletion of the VHL protein have recently been generated (Gnarra *et al.*, 1997). The heterozygous state appears to result in a normal phenotype in mice aged up to 15 months. The homozygous null condition is embryonically lethal, embryos dying at before day 12.5 *in utero*. This embryonic lethality has been shown to be a consequence of a failure of the development of the fetal side of the placental vasculature. This leads to placental haemorrhage, placental insufficiency and fetal death. VEGF is normally expressed at high levels in the developing placental vasculature but, interestingly, in the transgenic null mice there is deficient VEGF expression as part of the failure of placental angiogenesis. The relationship between VHL protein expression and the regulation of VEGF gene expression appears therefore to be more complex than at first suggested.

In summary, it has been shown that the von Hippel Lindau gene is mutated in the germ line of patients with the von Hippel Lindau syndrome and in the somatic cells of the kidney in sporadic renal cell carcinoma. The gene is an important gene during the development of the kidney and the placental vasculature. Reversal of VHL gene loss in *in vitro* experiments reverses many of the features of the transformed phenotype.

Hereditary papillary renal cell carcinoma (HPRCC)

Papillary renal cell carcinomas may, on occasion, be hereditary. This hereditary form is transmitted as an autosomal dominant pattern and the HPRCC gene has recently been shown to be located at 7q31.1–34. The most recent experiments have demonstrated mutations in the *c-met* gene (Schmidt *et al.*, 1997). This gene encodes the tyrosine kinase receptor for hepatocyte growth factor. Mutation in the c-met proto-oncogene has also been demonstrated in a number of sporadic papillary renal cell carcinomas. The mutations appear to lead to constitutive activation of the c-met tyrosine kinase. However, the simple heterozygous state for mutation appears to be insufficient to cause tumour formation and tumours only result when there is additional duplication of the mutated gene, usually as part of a whole chromosome 7 trisomy. Thus, papillary renal cell carcinomas associated with trisomies 7 and 17 appear to be a consequence, at least in part, of mutation and amplification of the c-met proto-oncogene.

In summary, our developing understanding of the genetics of renal cell carcinoma is leading to a more thorough understanding of the biology of tumour progression and the potential for therapeutic intervention.

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